Health and Medical Technologies

- Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers
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- Development of Reference Methods and Reference Materials for the Determination of Thyroid Markers and Other Non-Steroid Hormones
- Development of Reference Methods and SRM's for Toxic Species in Body Fluids
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- Microanalytical Technologies to Support NIH Measurement Programs
- Technical Procedures for a NIST Traceable Clinical Reference Laboratory Network
- Electrolytes in Human Serum

- **Program:** Health and Medical Technologies

Title: Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers

Authors: M.J. Welch, D.M. Bunk, N. Dodder, B.C. Nelson, M.B. Satterfield, L.T. Sniegoski, and S. S-C. Tai

Abstract: NIST has a strong commitment to promoting accuracy in health-related measurements and providing measurement traceability to the U.S. *in vitro* diagnostic (IVD) industry through development of reference methods and SRMs. Work has been completed on SRM 2921 Cardiac Troponin Complex (heart attack marker) and measurements have been completed for SRM 1955 Homocysteine and Folate in Human Serum (risk factor for heart disease and a substance that counteracts effects of homocysteine). NIST has completed development of methods for certain thyroid hormones in serum, is developing methods for additional hormones, and will be applying these methods to certification of an SRM for hormones in serum. Collaborative work with other NMIs is continuing on development of a reference method for C-reactive protein, a risk factor for heart disease. In response to a need for a better reference material for detection of kidney disease, work has started on a new frozen serum SRM for creatinine. Three important clinical SRM renewals were completed this year: SRM 1951b Lipids in Frozen Human Serum is a two-level material certified for cholesterol and triglycerides; SRM 956b Electrolytes in Frozen Human Serum is a three-level material certified for five electrolytes with reference values for ionized calcium; and SRM 965a Glucose in Frozen Human Serum is a four-level material with a much wider concentration range than the previous lot had. These new reference methods and SRMs will help the IVD industry meet new regulatory requirements for traceability.

Purpose: NIST has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. With issuance of the *in vitro* diagnostic (IVD) device directive by the European Union, it has become even more critical for NIST to develop new reference methods and SRMs to provide traceability for the US IVD industry so that this industry can maintain its strong position in European markets.

Major Accomplishments: SRM 2921 Cardiac Troponin Complex is now available to the clinical laboratory community. This SRM is a buffer solution containing a complex of heart muscle proteins, troponins I, C, and T and certified for the concentration of troponin I, a widely used marker for detecting that heart attacks have occurred. This new material will help improve the accuracy and reduce the variability of the clinical assays in use for detection of heart attacks.

Reference method development is complete and papers published for thyroxine (T4) and triiodothyronine (T3), markers for thyroid function. This work along with ongoing work to develop reference methods and a new SRM for non-peptide hormones is described in technical report #. Measurements have been completed for a new SRM for homocysteine (HCY) and tetrahydrofolic acid (FOL) in serum. This new material has three levels: (high HCY, high FOL; normal HCY and FOL; and low HCY, low FOL). HCY is considered a risk factor for heart disease and other diseases associated with oxidative damage, while FOL is a strong antioxidant, which is known to reduce the risk of neural defects in fetuses and is believed to counteract the

effects of homocysteine. This work is a collaborative effort between NIST and the Centers for Disease Control and Prevention (CDC), both of which provided measurements for certification. The incidence of kidney disease is rising rapidly in the U.S. Early detection of kidney disease and treatment can prevent kidney failure, but early detection depends on better measurements of kidney function. Serum creatinine is the preferred measurement, but existing methods provide varying results, so NIST is developing a new creatinine in frozen human serum SRM to address this measurement problem. As part of this work, NIST has developed a new, rapid isotope dilution LC/MS method for serum creatinine to replace the tedious isotope dilution GC/MS method used previously at NIST.

The renewal of SRM 956b Electrolytes in Frozen Human Serum is described in detail in Tech report #. SRM 1951b Lipids in Frozen Human Serum was certified for total cholesterol and triglycerides (triglycerides only and total glycerides) this past year. This material is available, but additional information on HDL- and LDL-cholesterol, additional heart disease risk factors, will be added once measurements are completed at CDC using their proposed reference methods for these analytes. For the renewal of SRM 965a Glucose in Frozen Human Serum, a new low-level material was added to address measurements of patients with severe hypoglycemia. The high level material was raised so that it better addresses measurements of patients with severe hyperglycemia.

Research is continuing on development of a reference method for another risk factor for heart disease, C-reactive protein (CRP). Modest increases in CRP have been linked to arteriosclerosis and the increased risk of heart attacks. NIST, working with scientists at the Laboratory of the Government Chemist (LGC) in the UK and the Physikalisch-Technische Bundesanstalt (PTB) in Germany, is using a proteomics approach to isolate characteristic peptides from CRP for measurement by LC/MS. An isotope labeled peptide will be used as an internal standard for this work. Research has also begun to investigate the quantitative potential of MALDI-TOF mass spectrometry for biomolecules. To explore this potential, studies are underway on promising approaches for quantification of transferrin, an important iron-transporting protein in blood. Research is also underway on measuring various selenium-protein (anti-cancer agents) and iron-protein (iron-transport) combinations in blood.

Impact: These new reference methods and the SRMs that they will be used to certify will provide critical traceability to the IVD industry and will help improve the reliability of routine clinical measurements. Better clinical measurements leads to better diagnoses, enabling earlier and more cost-effective treatments.

Future Plans: Work will be completed in FY05 for the new human serum-based SRMs for homocysteine/folate, hormones, and creatinine. Work will continue on new approaches for quantification of biomolecules, including peptides, proteins, hormones, and species containing inorganic elements.

Program: Health and Medical Technologies

Title: Development of Standard Reference Material (SRM) 2921, Human Cardiac Troponin Complex: A Primary Calibrator for Assays Used to Diagnose Heart Attacks

Author: D.M. Bunk and M.J. Welch

Abstract: With the assistance of the American Association of Clinical Chemistry (AACC), the International Federation of Clinical Chemistry (IFCC), and the manufacturers of clinical cTnI assays, NIST has developed a reference material to address the need for standardization of clinical human cardiac troponin I (cTnI) assays. Based on the results of two interlaboratory comparison studies, NIST Standard Reference Material (SRM) 2921 was prepared from the purified human cardiac troponin complex of the troponin T, troponin I, and troponin C subunits.

Characterization of the structures of the troponin subunits in SRM 2921 was performed by mass spectrometry and a certified value for the concentration of the troponin I subunit was determined by a variety of analytical techniques, including amino acid analysis. Manufacturers of commercial cTnI assays can now use SRM 2921 for quality control purposes and to establish direct SI-traceability of assay measurements, as well as for the value assignment of secondary reference materials.

Purpose: The clinical measurement of serum cardiac troponin I has become an important tool in the diagnosis of acute myocardial infarction and myocardial damage. Unfortunately, considerable variability in clinical cTnI assay results has been reported. Standardization of clinical cTnI measurements is needed to provide more reliability in the use of cTnI assays for the diagnosis of myocardial infarction and damage.

Major Accomplishments: After evaluating several different forms of troponin as candidate reference materials through two round robin studies, a human cardiac troponin complex was chosen as the most suitable material for SRM 2921. This choice was made after evaluation of the troponin preparation for purity, stability, its ability to provide harmonization of assay results, and the commutability of the material among the twenty commercial cTnI assays used in the studies.

Certification of the concentration of cTnI in SRM 2921 was accomplished by two analytical methods, including amino acid analysis. Through the amino acid analysis, SI-traceability of the cTnI concentration in SRM 2921 was achieved. Because of the inherent structural complexity of human proteins and because this complexity can have a substantial impact on immunoassay response, a thorough investigation of the chemical heterogeneity of cTnI and the other two troponin subunits, cTnT and cTnC, in SRM 2921 was performed. SRM 2921 was issued in May 2004 and has been well received by the *in vitro* diagnostic industry.

Impact: Manufacturers of commercial cTnI assays can use SRM 2921 for quality control, calibration, and to establish direct SI-traceability for assay measurements.

Future Plans: A commutability study is underway, involving nineteen cTnI assays currently on the market worldwide. Once accomplished, this demonstration of the commutability will put

SRM 2921 in compliance with ISO 15194 and eliminate the last hurdle for this new reference material to be acknowledged by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as a higher order reference material for use worldwide.

ADD FIGURE

Program: Health and Medical Technologies

Title: Development of Reference Methods and Reference Materials for the Determination of Thyroid Markers and Other Non-Steroid Hormones

Authors: M.J. Welch and S.S-C Tai

Abstract: NIST is developing reference methods and SRMs to support accuracy and traceability for hormone assays. New methods, based on LC/MS, have been developed for thyroxine, triiodothyronine, and cortisol [1-3]. Method development is underway for estradiol, progesterone, and testosterone. These methods will be applied to the certification of a new hormones in human serum SRM.

Purpose: Many life functions are regulated by hormones. When hormone levels deviate from normal, serious health consequences may result. Timely and effective treatments require accurate diagnoses of hormone levels. New reference methods and reference materials are being developed to support accuracy and traceability of clinical laboratory measurements of non-steroid hormones related to thyroid function and other metabolic processes.

Major Accomplishments: Reference method development has been completed and the methods published for thyroxine (T4) and triiodothyronine (T3), important markers for evaluating thryoid function, and cortisol, an important hormone in metabolism. These methods are all based on isotope dilution, liquid chromatography/mass spectrometry (LC/MS). Tandem mass spectrometry (MS/MS) is used to provide greater specificity in the measurements. These methods will be applied to the certification of new hormones in human serum SRM. This new SRM consists of two pools, one from normal adult males and one from normal, premenopausal adult females. Research is underway to develop new LC/MS-based reference methods for estradiol, progesterone, and testosterone, important hormones in development and reproduction. These methods will be applied to this new SRM.

Impact: Improving the accuracy of clinical assays for hormones will improve diagnoses and result in earlier treatments. The new methods and the SRM will help improve accuracy of these assays and will also provide high order reference systems for traceability.

Future Plans: Method development will continue and new SRMs developed as needed. This work should lead to capabilities for reference methods for synthetic pseudo-testosterone substances reportedly used by athletes to enhance performance.

References:

1. Tai, S. S. C., Sniegoski, L. T., and Welch, M. J., "Determination of Thyroxine in Human Serum by Isotope Dilution-Liquid Chromatography/Mass Spectrometry with Electrospray Ionization," Clin. Chem. 48, 637-642 (2002).

2. Tai, S.S-C., Bunk, D.M., White V, E., Welch, M.J., "Development and Evaluation of a Candidate Reference Method for the Determination of Total 3,3',5-Triiodothyronine in

Human Serum Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry," Anal. Chem, 76, 5092-5096 (2004).

3. Tai, S. S-C. and Welch, M.J., "Development and Evaluation of a Candidate Reference Method for the Determination of Total Cortisol in Human Serum Using Isotope-Dilution Liquid Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry," Anal. Chem., 76, 1008-1014 (2004).

Program: Health and Medical Technologies

Title: Development of Reference Methods and SRM's for Toxic Species in Body Fluids

Authors: S.J. Christopher, W.C. Davis, C.E. Bryan, and R.D. Day

Abstract: We have applied new and accurate analytical methods for the determination of toxic trace elements in whole blood using collision cell inductively coupled plasma mass spectrometry. New analytical instrumentation and a new methodology for detection of organometallic species in blood was developed and applied to the certification of methyl mercury (Me-Hg) at ultra-trace levels in SRM 966 Toxic Elements in Bovine Blood.

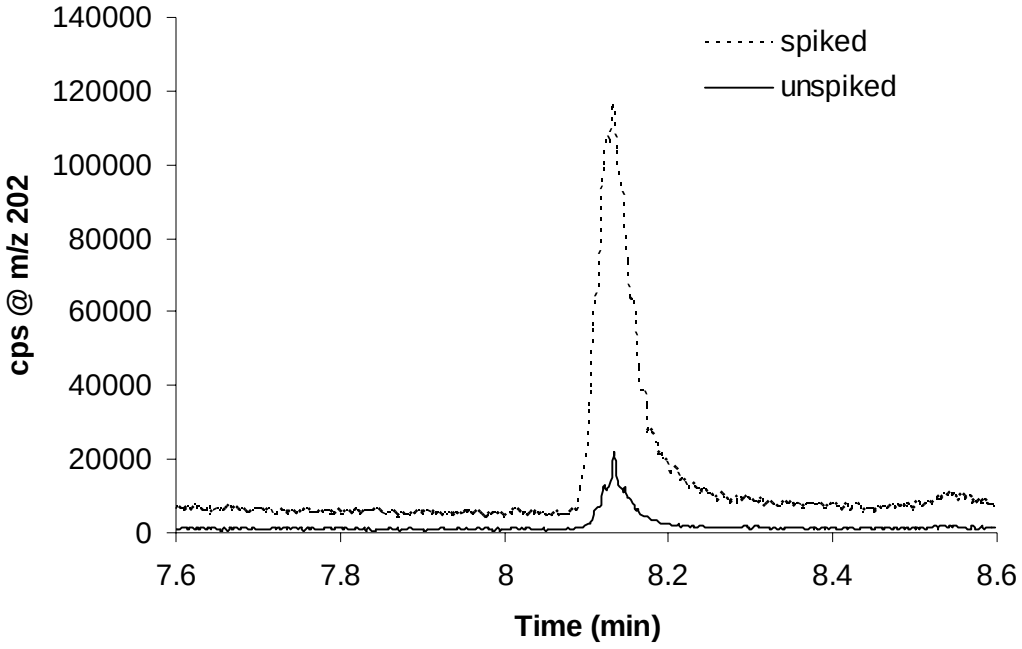
Purpose: The role of metals in health and medicine is all encompassing, with current research focusing on toxicology, drug delivery, degenerative diseases, imaging diagnostics and development of various classes of metal-based drugs possessing chemotherapeutic, anti-inflammatory, anti-diabetic or anti-microbial properties. Genomics and proteomics research will eventually lead to a better understanding of the role of metalloproteins in disease-proteins that may eventually serve as disease treatment targets. Fundamentally, all of this research is underpinned by the quality of analytical measurements in clinical samples like blood and urine. To address this we are developing accurate and sensitive analytical methods that can measure interference prone elements in a timely manner and producing clinical reference materials, which are closely aligned with industry, needs.

Major Accomplishments: One example involves the development of a new method for the determination of interference-prone trace elements (As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Rb, Se, Sr, V, Zn) in dolphin whole blood using collision cell inductively coupled plasma mass spectrometry (ICPMS). The method is based on gravimetric solution handling, and an internal standard ratio-based approach to quantification using the method of standard additions. A mixing scheme that facilitated preparing and running the samples in a "dilute and shoot" manner was developed to avoid a time consuming, high temperature and pressure sample decomposition. All clinical samples were prepared in a diluent consisting of several high purity chemicals: 1% 1-butanol + 1% Ammonium Hydroxide + 0.01% EDTA + 1% Triton X-100 + 1% nitric acid in balance high purity water (all by mass). The combined effects of this chemical suite serve to reduce the viscosity of the nebulized samples and ameliorate the differential ionization effects induced by carbon in individual analytical samples: 1-Butanol acts to reduce matrix effects by elevating the carbon in all matrices so that variable, sample-to-sample ionization effects are minimized, especially for As and Se. Ammonium hydroxide lyses red blood cells, EDTA prevents the loss of metals by precipitation or absorption, and Triton X-100 prevents blockage of the ICPMS nebulizer and torch injector tube. The calibration scheme was streamlined to avoid the numerous sample splits typically encountered in a standard additions experiment by using the slope of a blood standard additions curve to predict the concentration in unknown blood samples successfully, with any subtle matrix effects being compensated by the internal standards. A collision cell gas was used effectively to open isotopic channels for numerous interference prone elements including As and Se, by breaking apart background species generated in the plasma.

Another example involves the certification of methyl-mercury at low levels in SRM 966 Toxic Elements in Bovine Blood. This project involved development of new instrumentation and highly sensitive methods to address a critical problem for the CDC, which needs to benchmark Me-Hg measurements in clinical samples for various public health exposure efforts. A gas chromatograph (GC) was successfully coupled to an ICPMS via constructing a home built heated transfer interface, and solid phase microextraction (SPME) headspace sampling of microwave extracted samples was implemented to acquire the needed sensitivity to address this analytically challenging problem. Non-ideal extraction conditions can yield poor recovery or chemical transformation so several optimization studies were undertaken to gauge species liberation, degradation, and transformation during sample preparation and extraction, which are all circumstances presenting an obstacle for accurate quantification of any chemical species. Both methylmercury and inorganic mercury were monitored in SRM 966 and quantified during the method testing phase and the sum of the methyl and inorganic mercury species were in excellent agreement with certified values for total Hg. The method of standard additions provided the means for Me-Hg quantification. Detection limits for Me-Hg using the SPME-GC-ICPMS method were 4.2 pg/g, while identical headspace sampling and chromatographic conditions allowed for separation and detection of organo-tin species at the fg/g level.

Impact: The program examples highlighted here show that CSTL's measurement capabilities are well positioned to benchmark accurately the next generation of measurements for metals in clinical samples, regardless of chemical form or whether the samples are derived from human or non-human sources. The new calibration approaches developed for dolphin blood health assessments proved to be robust and applicable to similarly matched matrices of human and animal origin, suggesting that current-issue clinical SRMs can be used to benchmark whole blood measurements from any similar matrix. A similar approach could be developed for urine measurements.

Future Plans: Work will be dedicated to applying GC-ICPMS to solve ultra-trace speciation problems involving Hg and Sn chemical species and isotope dilution methods for species quantification will be explored. Liquid chromatography-collision cell-ICPMS methods will be used to certify organoarsenic compounds in a urine SRM. The method of double isotope dilution will be applied to a pharmacokinetics study (funded by S.C. Sea Grant through the College of Charleston). Staff from the FDA will assist with modeling the dynamics of orally administered MeHg in the blood of diamondback terrapins. Enhanced performance of the pharmacokinetics models will result from high accuracy measurements and the ability to quantify dose and background levels of blood Hg among replicates independently, eliminating model noise arising from individual variability.



Chromatogram for the spiked and unspiked extracted methylmercury in SRM 966 Toxic Elements in Bovine Blood.

Program: Health and Medical Technologies

Title: Standards for Fluorescence Microarray Analyses

Authors: G.W. Kramer, A.G. Gaigalas (831), and P.C. DeRose

Abstract: A working group representing all of the U.S. manufacturers of microarray scanners has asked NIST to develop standards for the quantitation of fluorescent microarray analyses. Specifications for a series of physical artifacts have been developed during several NIST workshops and monthly teleconferences. Work continues to find suitable fluorescent materials, to identify appropriate fabrication methods, and to create instrumentation for certifying the fluorescence of such standards.

Purpose: DNA microarrays, also known as DNA or gene chips, have become important tools for gene expression analyses and are poised to revolutionize clinical diagnostics and enable personalized medical care—where treatment can be individually tailored to a specific person through genetics-based diagnoses. The related protein microarrays show great promise for pharmaceutical drug discovery research as well as clinical diagnostic tools. For microarrays, single-stranded genetic or protein material (probes) is bound in an array to a surface the size of a standard microscope slide permitting tens of thousands of molecular reactions to be tracked in parallel when an analyte solution is washed over the array. To accommodate this many sites in such a small area, the individual spots of genetic material or protein must be very small, commonly on the order of 10 to 100 micrometers in diameter. Target DNA or protein in the analyte solution are labeled with a fluorescent dye and allowed to interact with the probes bound on the surface of the array. A favorable interaction leads to fluorescence signal from the appropriate spots. A device called an array scanner or reader detects which spots in the array fluoresce and how much.

Today, there is no consensus method for microarray assays; there are multiple technologies for fabricating microarrays ranging from nano-pipetting to pin-printing to photolithography, and there are several types of scanners. The method variations and uncertainties are so great currently that the biological information obtained from microarray assays is often a function of the method and equipment used, and comparisons of results generated by differing technologies is not possible. If artifact standards for microarray assays can be created and certified, assay quantitation can be improved, results will be intercomparable, and the measurements can be made traceable ultimately back to the SI. The purpose of this project is to develop such standards in collaboration with the manufacturers of microarray readers.

Major Accomplishments: This project began with a request from the scanner industry for NIST to develop standards for this field. A technical workshop on fluorescence standards for microarray assays co-sponsored by Agilent Technologies and NIST's Biotechnology and Analytical Chemistry Divisions was held in late 2002 and attended by representatives from the major array reader manufacturers. The purpose of the workshop was to develop technical specifications for fluorescence intensity, uniformity, and detection limit standards for the calibrating and validating microarray readers. Several parameters such as the excitation and emission wavelengths of the fluorescent tags seemed to be common across the industry;

however, there were diverging views on other specifications such as spot size and the form factor of the array. Following the initial technical workshop, the participants worked out many issues concerning the types of standards, intensity levels, form factors, etc. in teleconferences. It was agreed that unpatterned artifacts for each of two colors (similar to the dyes Cy3 and Cy5 that are commonly used now) would be developed. One set of artifacts with fluorescence intensities in the mid-to-high range would serve as uniformity/homogeneity standards and to measure signal-to-noise ratios for bright features, while a second set of materials would be developed with low fluorescence levels to serve as detection limit standards and to measure signal-to-noise ratios for dim features. A second technical workshop was held at NIST in the spring of 2003 to come to agreement on many of the physical factors and to begin the search for appropriate fluorescent materials and application processes to fabricate the standards.

Through quarterly teleconferences, the group continues to refine parameters and to search for suitable materials. Recently, NIST began collaborating with private sector firms in the search for candidate materials. We have begun to examine several glass technologies with the help of R&D personnel from Schott Glass, Inc. (Duryea, PA), and we have recently entered into a CRADA with Evident Technologies, Inc, (Troy, NY) to study the use of nanocrystal composites. The working group has also developed a set of procedures and specifications for testing the suitability of candidate materials. The primary difficulty is finding materials with acceptable spectral characteristics that can withstand the high laser light intensity without photodegrading.

Impact: The development of artifact standards for quantitative fluorescence assays on microarrays is a first step in multi-requirement process for standardizing microarray assays. However, it is essential that this process go forward to allow the use of microarrays in clinical settings instead of just research venues. Microarrays have a very promising future, not only in the clinical/biotech/pharmaceutical applications that are being developed now, but also for general chemical analyses. The impact of microarray technologies on the future of analytical chemistry could be huge.

Future Plans: We are currently searching for suitable materials to develop into the artifacts and are building the instrumentation necessary to make the certification and stability measurements. Once these issues are behind us, and we have viable materials, we can concentrate on developing standard protocols for their use. We have discussed these issues with the appropriate ASTM and NCCLS committees. It is likely that we will work jointly with both organizations to develop these documents.

Program: Health and Medical Technologies

Title: Microanalytical Technologies to Support NIH Measurement Programs

Authors: L.E. Locascio (839), M. Gaitan (812), D. (812), N. Morgan (NIH/OD/ORS), P. Smith (NIH/OD/ORS), T. Pohida (NIH/CIT), T. Phillips (NIH/OD/ORS), E. Perruccio (NIH/NEI), P. Becerra (NIH/NEI)

Abstract: We recently established collaborations with NIH scientists for the purpose of enhancing medical research using microfluidics and microengineering. There are two distinct ongoing projects with different teams at NIH whose research involves the following:

- *Study of the immune response to Herpes Papilloma Virus (HPV)*
- *Study of vision-related diseases involving defective neuronal differentiation or cell survival*

For the first project, we are developing chip-based microfluidic devices to be used in multi-analyte immunoaffinity capture and detection of proteins related to HPV in cervical secretions. The second project involves patterned immobilization of mammalian retinal cells using microfluidic channels to guide their growth and to facilitate observation of their behavior under different conditions and treatments.

Purpose: The purpose of this collaborative effort is to fabricate microsystems based on MEMS and microfluidics to support and improve the measurement capabilities of medical researchers at the NIH laboratories.

Major Accomplishments:

Project 1. Human Papilloma Virus: This work involves an epidemiological study of the immune response to the Herpes Papilloma Virus (HPV), for which the simultaneous isolation of multiple proteins from microliter samples of cervical secretions is required. For this purpose, we are developing a microfluidic system capable of multi-analyte detection in a single small volume sample by performing multiple sequential heterogeneous immunoassays on chip. Microfluidic channels designed in a serpentine pattern are fabricated at NIST using silicon and glass substrates as shown in Figure 1. Different antibodies are then covalently immobilized to each

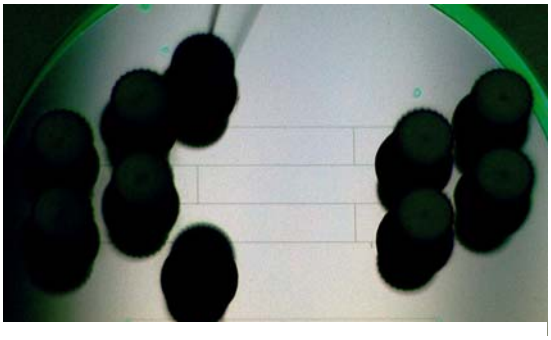


Figure 1a. Microfabricated silicon/glass device with 4 different arms for antibody

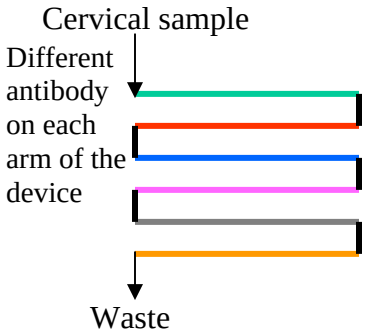


Figure 1b. Schematic diagram of device with 6 arms for measurement of 6 independent analytes.

separate arm of the chip so that the system is capable of measuring a number of analytes equal to the number of arms. The channel device architecture has several advantages over existing array technology including the fact that the device is reusable, and the captured proteins can be extracted after measurement with their biological activity intact. We are designing the system so that the basic methodology can be applied to many different applications relevant to clinical and biological research at both institutions.

Project 2. Vision-related disease: There exist a number of techniques that have been used for patterning cells on surfaces. The strategy often used to adhere single (mammalian) cells involves immobilization of extracellular proteins onto the surface for further adhesion of the cells by interaction with these proteins. In this work, immobilization of rat retinal epithelial cells is accomplished using micropatterned polyelectrolyte multilayer (PEM) -coated surfaces. PEMs were deposited in discrete lines using a (poly)dimethyl-siloxane (PDMS) microfluidic network on top of a flat PDMS slab. The layers were formed by sequentially flowing the polyions throughout the microfluidic network. Retinal cells, seeded on flat PEMs/PDMS surfaces adhered and grew on the PEM areas preferentially as shown in Figure 2. Cells were allowed to grow for up to two weeks showing the pattern delineated by the PEMs. We believe this approach could provide a useful and simple tool to pattern single cells or cell networks in specific locations to monitor neural activity of retinal cells as they are exposed to different stimuli.

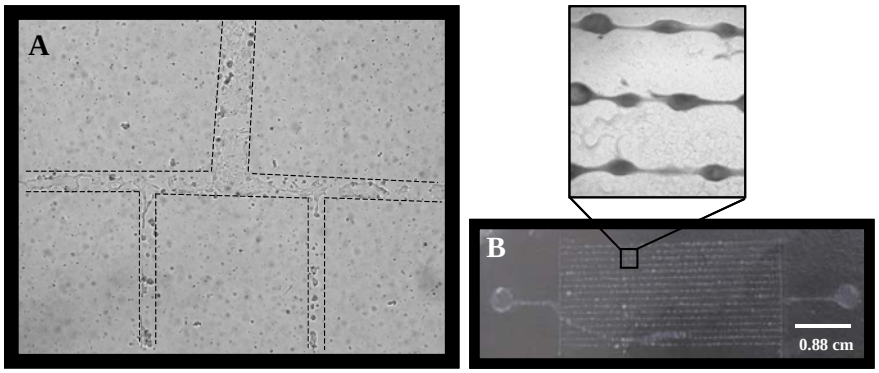


Figure 2. A) Overnight growth (~18hr) of retinal cells on poly(ethyleneimine). Cell growth is observed on the polycation surface areas (within the dotted black lines). B) Two weeks cells' growth on poly(ethyleneimine). Cells are covering all the polycationic area.

Impact:

We have found these partnerships with NIH to be very beneficial to our research at NIST. In the last 8 years, we have developed strong microfabrication expertise and capabilities in-house, and have recently been working toward the application of microtechnologies to solving critical problems in environmental monitoring, forensics, nanotechnology, and now, healthcare. We believe that micro- and nanotechnology can be of great use to those involved in clinical research allowing for very elegant small volume sample manipulation and treatment as demonstrated by our successes in the first years of these projects.

Future Plans:

The goal of the first project in the next year is to demonstrate multianalyte capability and determine the sensitivity and detection limits of assays performed in this manner. In the next year we also plan to continue work with retinal cells to examine their viability and growth on patterned PEMs surfaces.

Program: Health and Medical Technologies

Title: Technical Procedures for a NIST Traceable Clinical Reference Laboratory Network

Authors: G.C. Turk, S.E. Long, and D.L. Duewer

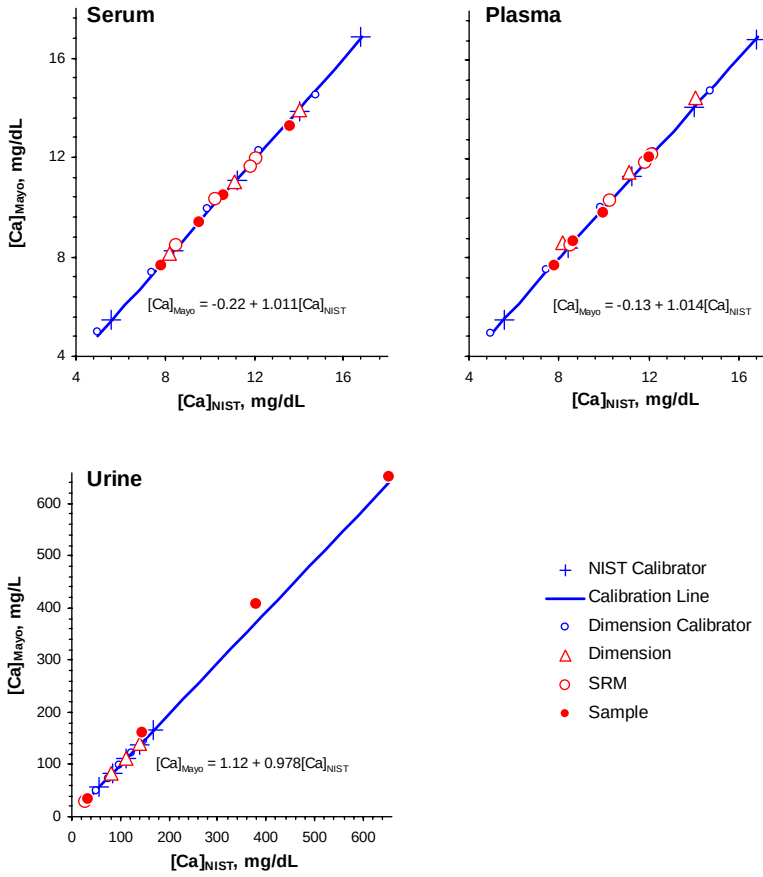
Abstract: In December of 2003 the European Union Directive on In-Vitro Diagnostics (IVD) went into effect mandating IVD traceability to "higher order" standards and methods. Sales of NIST electrolyte in serum SRMs have increased dramatically in response, as has the needs of U.S. IVD manufacturers for higher-order reference measurements. With the goal of building an efficient infrastructure for providing clinical reference measurements for IVD manufactures, NIST entered into a collaboration with Mayo Clinic and Dade-Behring, Inc. to test technical procedures that might be used to implement a NIST Traceable Clinical Reference Laboratory Network. Such a network could provide the IVD industry with reference measurements traceable to NIST standards in a timely and efficient manner. The specific test conducted involved the measurement of calcium at various levels in blood serum, plasma, and urine. A subset of real samples of serum, plasma, and urine were measured both at NIST and the Mayo Clinic, using NIST calibration and validation SRMs, in order to establish an equivalence between NIST isotope dilution mass spectrometry measurements and measurements performed at Mayo Clinic. Initial results have been encouraging, and could potentially lead to the development of a network of laboratories with an expanded analyte and matrix coverage.

Purpose: To design and test practical measurement procedures that could be used to establish traceability of non-NIST clinical reference measurements to NIST standards and measurements.

Major Accomplishments: This study was designed around the analysis of individual patient samples that were split and analyzed by both NIST and Mayo Clinic. The patient samples were pre-screened to cover the full range of calcium concentration for which the methods have been designed. All patient identification information was disassociated with the samples for this study. Further validation of the methods was provided by the analysis of NIST matrix SRMs by both NIST and Mayo. These SRMs include SRM 956a (Frozen Human Serum) and SRM 2670a (Freeze Dried Urine). In addition, both NIST and Mayo Clinic analyzed Candidate SRM 956b (Frozen Human Serum), which had not yet been certified, and for which the Ca concentration was unknown to Mayo Clinic at the time of the analysis.

The protocol called for Mayo Clinic to use their normal calibration procedures, based on their in-house supply of calibrator solutions. They also prepared a set of NIST Calibrators by dilution of SRM 3109a (Calcium Solution). Five NIST calibrators were prepared at concentrations similar to those of the Mayo Calibrators. Mayo used their normal setup and calibration procedures and then analyzed the samples included in the study using a run order supplied by NIST. Each sample, including the NIST calibrators, was treated as an "unknown", and value assigned based on the normal Mayo calibration procedure with their in-house set of calibrators. The ratio of the determined values of the NIST calibrators relative to the prepared values using the NIST certified values was calculated. This value, and associated uncertainty, was then used to convert all of the Mayo determined values of the test samples (traceable to the Mayo calibrators) to

values traceable to the NIST calibrators and SRM 3109a. The conversion shift was small, but significant. The comparisons between NIST measurement results and those of the Mayo Clinic are illustrated graphically in the figure below for each sample matrix.



Impact: A NIST Traceable Clinical Reference Laboratory Network would become an important component within the clinical chemical measurement infrastructure, with a positive impact on the accuracy of such measurements and on the quality and cost effectiveness of our healthcare system.

Future Plans: Discussions are ongoing with our collaborators to evaluate the effectiveness and practicality of the approach used in this exercise. Possibilities for the future include formal development of the proposed laboratory network, and expansion to other analytes and matrices.

Program: Health and Medical Technologies

Title: Electrolytes in Human Serum

Authors: S.E. Long and K.E. Murphy

Abstract: We have developed new analytical methods utilizing inductively coupled plasma – mass spectrometry (ICP-MS) for the determination of electrolytes in blood serum and have employed these in the certification of a renewal material, SRM 956b, Electrolytes in Frozen Human Serum. SRM 956b is widely used for the calibration and standardization of automated analyzers and electrolyte measurement systems employing ion selective electrodes. The new methods are faster, and therefore more efficient than previous methods, which were based on thermal ionization mass spectrometry (TIMS). A new ICP-MS instrument fitted with both collision cell and shielded-plasma technology for reducing mass spectral interferences has recently been installed in our laboratory. The new system will complement the existing instrumentation and will provide enhanced capabilities for the determination of problematic clinical analytes such as calcium and potassium.

Purpose: Accurate and expedient clinical assessment of patient health status is often critically dependent on the quality of clinical testing measurements. For example, a recent report, commissioned by NIST, on the laboratory testing of calcium [1] concluded that the potential economic impact on the health-care system of even a modest analytical bias could be in the range $60 to $199M resulting from the need to provide (often unnecessary) follow-up patient testing. The availability of appropriate high-quality reference materials to provide a traceability basis for such clinical measurements is an important requisite. This was recognized by the European Union, which recently introduced the In-Vitro Diagnostics (IVD) Directive, which came into effect in December 2003. Electrolytes are undoubtedly the most commonly measured analytes in the clinical laboratory, and therefore the SRM 956 series, which provides certified values for electrolytes in blood serum, provides an extremely important resource to the clinical measurement community. At the time of the implementation of the IVD Directive, sales of SRM 956 increased dramatically. To support the continuing regeneration of this material, it is necessary to develop and maintain higher-order analytical methods, which utilize state-of the-art measurement technology.

Major Accomplishments: New methods using isotope dilution ICP-MS have been developed for the determination of lithium, total calcium and potassium in human serum and have been used to certify SRM 956b, Electrolytes in Frozen Human Serum. Certification of five electrolytes, namely lithium, potassium, total calcium, sodium and magnesium in three levels comprising SRM 956b, has been completed. All of the certified values were obtained using ICP-MS, which has permitted a more efficient certification strategy. Certification measurements on the previous issue (956a) relied heavily on TIMS analytical methods. While TIMS can provide unsurpassed accuracy and repeatability, it is an extremely slow technique. The new methods for calcium and potassium are based on shielded (cool) plasma ICP-MS. Significant effort was made to develop a robust method for the determination of lithium by ICP-MS. Lithium has not been routinely measured using this technique because of severe problems with instrument mass bias

drift and significant inter-sample memory effects. In the new method these effects have been almost eliminated by the careful optimization of ICP-MS instrument conditions.
The analytical methods currently used for electrolyte measurements have been compiled into a methods manual, which we intend to publish both as a NIST 260 Series report and as an on-line document available to external users of the NIST web site.
A quadrupole ICP-MS instrument, configured with both collision cell and shielded plasma technology to reduce or eliminate spectral interferences has been installed in the Analytical Chemistry Division. It is anticipated that this will provide new capabilities for the determination of clinical analytes, which have been difficult to measure accurately using traditional instrumentation.

Impact: The development of new analytical methods for electrolytes in clinical materials has provided significant enhancements to ACD measurement capabilities and has improved the efficiency of SRM certification activities. The new methods have been used to renew SRM 956b, which is an important clinical SRM and which should provide valuable traceability support for both domestic and European IVD markets.

Future Plans: Development work on new analytical methods using collision cell ICP-MS for the determination of electrolytes and other important clinical analytes in serum and urine will be actively investigated.

References:

1. Gallagher M.P, Mobley L.R., Klee G.G and Schryver P., "RTI Planning Report 04-1, The Impact of Calibration Error in Medical Decision Making," April 2004.